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# STRUCTURE OF VIRIDITOXIN, A TOXIC METABOLITE OF ASPERGILLUS VIRIDI-NUTANS

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During the screening of Aspergillus strains for toxin producers, a compound toxic to mice was extracted from mycelia of A. viridi-nutans Ducker and Thrower NRRL 4365 grown in Raulin-Thom medium (1). After the compound, trivially named, viriditoxin, was extracted with chloroform:methanol (80:20) and isolated by chromatographic separation on activated magnesium silicate columns with chloroform:acetonitrile:formic acid (95:4:1) as eluting solvent, the purified fraction was readily crystallized from cold benzene. The yield was 0.7% based on wet mycelial weight. A suspension of the green substance in propylene glycol (I.P.) had an $LD_{50}$ of 2.8 mg/kg in 20-g mice.

Elemental and mass spectral analyses of viriditoxin (mp 242-245° dec, $[\alpha]_D^{28°}$ -202° in $CHCl_3$) established the molecular formula as $C_{34}H_{30}O_{14}$. The IR spectrum exhibited a band at 1740 $cm^{-1}$ attributed to an ester carbonyl and a band at 1635 $cm^{-1}$ that shifted to 1720 $cm^{-1}$ upon acetylation and was assigned to a hydrogen-bonded six-membered lactone. Because the NMR spectrum (2) of viriditoxin showed only 15 protons, the structure for the new toxin was probably that of a symmetrical dimer. Two exchangeable protons appeared at $\delta$9.72 and 13.70. The position of these peaks indicated that phenolic hydroxyl was present and, in addition, that the lowest field hydroxyl was hydrogen-bonded to a carbonyl function. Methoxyl resonances at $\delta$3.66 and 3.74 account for an additional six protons. Two aromatic protons appear as singlets at $\delta$6.24 and 6.78. When viriditoxin was acetylated, the aromatic proton resonances were shifted to lower field ($\delta$6.79, 7.11) and peaks representing the acetate methyls were observed at $\delta$2.48 and 2.51. The rather large downfield shifts of 0.55 and 0.33 ppm were indicative of a para relationship between the hydroxyl groups and the aromatic protons.

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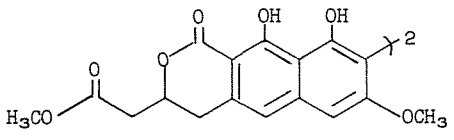
The five remaining protons are in a system of two methylenes interacting with the same methine proton. The methine proton appears as a multiplet centered at $\delta 4.96$, while the methylene resonances overlap each other at $\delta 2.76$ and 2.81. The methylene protons centered at $\delta 2.81$ have the same chemical shift and appear as a doublet, which contrasts with the eight-line pattern for the methylene resonance centered at $\delta 2.76$. This pattern is typical of the AB portion of the ABX spectrum. Consideration of the NMR spectrum leads to structure 1 as proposed for viriditoxin.

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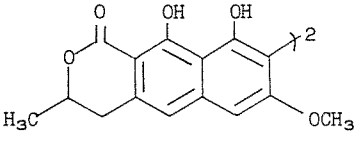


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Blank (3) and coworkers isolated a pigment, vioxanthin, to which they assigned structure 2. The spectral data for the two compounds were, as might be expected, similar. The NMR spectrum of vioxanthin shows two exchangeable protons at $\delta 9.70$ and 13.84 and a methine resonance at $\delta 4.70$. In addition, the carbonyl region of the infrared spectrum showed a peak at 1640 $cm^{-1}$ which shifted to 1725 $cm^{-1}$ upon acetylation. A comparison of the UV spectral data further demonstrates the similarity between structures 1 and 2. UV maxima occur at 266 ($\varepsilon$82,000) and 380 nm ($\varepsilon$22,500) in structure 1 and at 268 ($\varepsilon$50,000) and 380 nm ($\varepsilon$14,000) in structure 2. After acetylation the respective maxima were found at 268 and 335 nm (viriditoxin) and at 262.5 and 320 nm (vioxanthin).

Acknowledgment

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REFERENCES

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2. NMR spectra were recorded with a Varian HA-100. The mention of firm names or trade products does not imply that they are recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned. All spectra were run in $CDCl_3$ with tetramethylsilane as the internal reference.
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